

AMENDMENT

Amendments to the Specification:

Please amend the paragraph beginning on page 1, line 11 as follows:

This application is a divisional of U.S. Patent Application Serial No. 09/910,552, filed July 23, 2001, now U.S. Patent No. 6,642,354, which is a divisional of U.S. Patent Application Serial No. 09/494,822, filed January 31, 2000, now abandoned, which is a continuation of U.S. Patent Application Serial No. 08/925,002, filed August 27, 1997, now U.S. Patent No. 6,048,257, from which applications priority is claimed pursuant to 35 U.S.C. §120; and is related to provisional patent application serial no. 60/025,799, filed August 27, 1996, from which application priority is claimed under 35 U.S.C. §119(e)(1) and which applications are incorporated herein by reference in their entireties.

Please amend the paragraph beginning on page 58, line 15 as follows:

50 µl aliquots of various dilutions of the monoclonals were added to wells of replicate plates containing either 50 µl of Diluting Buffer or 50 µl of Diluting Buffer containing 50 µg of soluble NAc-MenB PS per ml (for a final inhibitor concentration of 25 µg/ml). The plates were then covered and incubated overnight at 4°C. On the following day, the wells were washed five times with cold Washing Buffer and then incubated for 3 hours at 4°C with 100 µl/well of alkaline phosphatase conjugated anti-murine IgG, IgM and IgA polyclonal antibodies (Zymed ZYMED) diluted 1:2000 in Diluting Buffer. The plates were then washed with cold Washing Buffer, and 100 µl of freshly prepared substrate (p-Nitrophenyl phosphate, Sigma SIGMA) diluted to 1 mg/ml in Substrate Buffer was added to each well. Absorbance values at 405 nm were measured after approximately 30 minutes.

Please amend the paragraph beginning on page 65, line 24 as follows:

Control antibodies in the assay included: (1) an IgG monoclonal antibody of irrelevant specificity (VIIG10, as a negative control); (2) an IgM anti-polysialic acid monoclonal antibody

(2-1B, as a positive control); and (3) an anti-CD56 monoclonal antibody specific for the protein backbone of NCAM (Immunotech, Marseille, France). Blocking Buffer (2 ml) was added to each reaction tube, and the tubes were centrifuged at 1000 rpm in the ~~Sorvall~~ SORVALL RT-600B for 6 minutes at 20°C. Following centrifugation, the supernatant was aspirated off and the cells incubated for 1 hour at ambient temperature with 150 µl of fluorescein isothiocyanate (FITC)-conjugated F(ab')₂ fragment goat anti-mouse IgG (H+L) (diluted to 4 µg/ml) (Jackson Immune Research, West Grove, PA). After washing with Blocking Buffer, 400 µl of 0.25% formaldehyde in PBS buffer (50 mM sodium phosphate, pH 7.0, 150 mM sodium chloride) was added to the cells, and the cells were analyzed by flow cytometry using a FACSCAN™ cell sorter (Becton-Dickinson, Mountain View, CA).

Please amend the paragraph beginning on page 68, line 6 as follows:

Bacterial Strains: *Neisseria meningitidis* group B strain IH 5341, a human patient isolate with MenB:15:p1.7, 16 phenotype, plus 1 to 2 additional other group B bacterial strains (e.g. M355; B:15:P1.15) were used. All bacteria strains were rat passaged five times and stored in skim milk at -70°C. For each experiment, a fresh inoculum was taken from the stock and cultivated on gonococcal (GC) medium base (GC-agar II Base, Becton Dickinson, Mountain View, CA) supplemented with ~~IsoVital~~X ISOVITALEX, L-tryptophan and hemoglobin. After incubation overnight at 37°C in 5% CO₂, several colonies were inoculated into a culture flask containing 20 ml of brain-heart infusion broth and incubated at 37°C in a rotatory shaker at 150 rpm until the optical density (Klett 90) corresponded to 10⁸ cfu/ml. The cultures were then diluted in phosphate buffered saline (PBS) corresponding to 10⁶ cfu/ml for use. The actual number of viable bacteria in a challenge dose was determined by counting the cfu after serial dilution of the suspension in PBS and plating on proteose peptone agar.

Please amend the paragraph beginning at page 79, line 7 as follows:

Preparation of OMP Vesicles. OMP vesicles were prepared from the capsular-deficient mutant strain of *Neisseria meningitidis* Group B (Strain M7), using a combination of the

techniques described by Lowell et al. (1988) *J. Expt. Med.* 167:658-663 and Zollinger et al. (1979) *J. Clin. Invest.* 63:836-848. In brief, *Neisseria meningitidis* strain M7 (a noncapsular mutant strain derived from NmB), from an overnight culture on chocolate agar plates incubated at 37°C, was used to inoculate two 500 ml flasks of sterile Frantz medium (10.3 g of Na₂HPO₄, 10 g of casamino acids (Difco, Detroit, MI), 0.36 g of KCl, 0.012 g of cysteine-HCl (Sigma SIGMA, St. Louis, MO), and 25 ml of 40% glucose-40 mM MgSO₄ (Sigma SIGMA, St. Louis, MO) in 1L of water, pH 7.4). The bacteria were grown from an initial OD of 0.1 - 0.2 to log phase (OD of 0.75 - 0.85) on a shaker at 180 rpm for 6-8 hours. The bacteria were inactivated with 0.5% phenol solution for one hour at room temperature. The cells were harvested by centrifuging for 30 minutes at 3000 x g. The supernatant was decanted, and the cells were washed twice with PBS. The resultant pellet was stored at -20°C.

Please amend the paragraph beginning on page 79, line 28 as follows:

The bacteria were then resuspended in 15 ml buffer containing 0.05 M Tris-HCl, 0.15 M NaCl and 0.01M EDTA (pH 7.4), and then warmed to 56°C for 30 minutes. After cooling to room temperature, the suspension was sheared in a ~~Polytron~~ POLYTRON (Kinematica GmbH., Luzern, Switzerland) at full speed for 3 minutes and then centrifuged at 16000 x g for 15 minutes. The resulting pellet was resuspended with 10 ml buffer (500 mM sodium chloride, 50 mM sodium phosphate), and treated with 5 ml of Detergent Solution (10% sodium deoxycholate (DOC) (Calbiochem, La Jolla, CA), 0.15 M glycine (Biorad, Hercules, CA) and 30 mM ethylenediaminetetraacetic acid (EDTA) (SIGMA, Saint Louis, MO). The suspension was centrifuged at 16,000 x g for 15 minutes. The supernatant was then collected and centrifuged at 100,000 x g for 2 hrs. A pellet containing the outer membrane protein preparation was resuspended in 10 ml of water and stored at 4°C.